

Collection Techniques for Fish Ageing Structures Northwest Region

NWSI Technical Report TR-73 Revised

September 2004

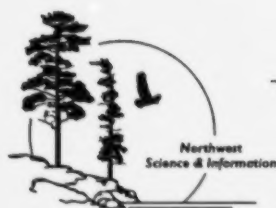
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NWSI Technical Report TR-73 Revised

September 2004

by

S.E. Mann



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Disclaimer

The views, conclusions and recommendations are those of the author(s) and should not be construed as either policy or endorsement by the Ontario Ministry of Natural Resources.

Table of Contents

	Page
Acknowledgement	iii
Disclaimer	iii
List of Tables	v
List of Figures	v
List of Appendices	vi
Introduction	1
Sampling Techniques for Calcified Structures	1
Otoliths	2
Dorsal Method	4
Ventral Method	4
Cleithrum	5
Opercular Bone	7
Spines	8
Fin Rays	9
Scales	9
Data Records	11
Shipping	12
Summary	12
Literature Cited	13
Appendices	16

List of Tables

	Page
Table 1	2
Structures to be collected from various fish species. Structures are listed in order of ageing reliability.	
Table 2	11
AGEST section of the OFIS Data Dictionary.	

List of Figures

	Page
Figure 1	3
Anatomy of vestibular apparatus. (A) Otoliths within the labyrinth systems of representative teleost and ostariophysean fishes. (B) Dorsal view of the vestibular apparatus as it sits in a typical teleost. The top of head is cut away. Ast = asteriscus; Lag = lagenar vestibule; Lap = lapillus; Sac = saccular vestibule; Sag = sagitta; Utr = utricular vestibule. (Secor <i>et al.</i> 1991)	
Figure 2	4
(A) Location of the dorsal incision for the removal of otoliths and (B) the top view of the brain and location where the forceps should be inserted. (Scarnecchia 1987)	
Figure 3	5
Schematic diagram of the ventral view of the parasphenoid bone. (Norden 1961)	
Figure 4	5
Location of incision on the ventral surface of the parasphenoid bone of a walleye. (Photo by Susan Mann)	
Figure 5	6
Method of removing cleithra from northern pike and other esocids. (Casselman 1979)	
Figure 6	7
Photo of a cleithrum that was improperly removed from a pike. Notice the pectoral fin ray is still attached as well as a large amount of muscle and connective tissue. (Photo by Susan Mann)	
Figure 7	7
Diagram of a perch to show the location of the opercular bone. (Le Cren 1947)	
Figure 8	8
Photo of an opercular bone taken from a walleye. Age estimate is 15 years. (Photo by Susan Mann)	
Figure 9	8
Location of the dorsal spines used for age assessment on a spiny-rayed fish. (Drawing take from Scott and Crossman 1973)	

Figure 10	9
Location of the pectoral fin spine used for age assessment on bullheads and catfish. (Drawing taken from Scott and Crossman 1973)	
Figure 11	10
(A) shows the position where the fin should be removed by cutting through or close to the ridge. (B) shows the two fin ray elements for each fin ray (Beamish 1981).	
Figure 12	11
Location of scale removal from: walleye and other percids; bass and other centrarchids; trout and other salmonids; and pike and other esocids. (Drawing by Susan Mann)	

List of Appendices

Appendix A	16
Examples of ageing error using the scale method (Beamish and McFarlane 1987).	
Appendix B	17
Comparison of preparation and ageing time for 100 samples of each structure at the Northwest Region Ageing Laboratory.	
Appendix C	17
Supplies and supplier.	

Introduction

This manual provides guidelines for collecting, storing, recording, and shipping calcified structures to the Northwest Regional Fish and Wildlife Ageing Lab in Dryden, Ontario. The proper collection of calcified structures will assist in attaining more precise ages and will also reduce preparation time in the laboratory.

When planning any fisheries project, knowing what calcified structure (i.e. scales, otoliths, cleithra, fin rays, or spines) to collect for each target species and how to collect them is as important as the sampling design, survey equipment, type of biological data needed, and analysis method of data, etc. Until recently, scales were almost solely collected for age assessment. Unfortunately, the method was seldom validated (Beamish and McFarlane 1983) and investigators did not consider the possibility that fish could live for many years with little or no growth. There are a number of recent studies that clearly show that scales underestimate the age of older fish (Appendix A). If incorrect ages are assigned to older fish as a result of using the scale method, then there is an accumulation of ages at the point where the method breaks down. This results in a serious overestimate of production (Beamish and McFarlane 1987).

Other structures, such as the sagittae (otoliths), spines, fin rays, and cleithra have been shown to retain their physical structure during stressful periods of a fish's life and thereby more accurately record true age.

Sampling Techniques For Calcified Structures

The calcified structures available for collection from each fish species in the Northwest Region are listed in Table 1 in order of reliable ageing. **During sampling, at least two different types of structures should be collected from each fish and placed in a scale envelope.** If the first structure does not yield a confident age assessment, a backup structure is available in the scale envelope. Circle which "age structures" (e.g. 2=scales, 4=pectoral fin, A=otolith, etc.) were collected on the front of the envelope.

Before going out into the field and collecting samples, decide how many fish samples should be taken. Taking too many fish samples can waste time and resources, both in collection and data analysis. On the other hand, taking too few fish samples may make the study meaningless, or even worse, lead to errors in interpretation (Eckblad 1991, Fruetel 1996).

Consider the choice of structure in terms of the type of information that it can yield; that is, otoliths and spines provide reliable ages but are not easy to use for back calculations whereas opercular bones and scales can be used for both (Bev Ritchie, personal communication). If the fish are going to be sacrificed, I strongly recommend collecting otoliths for ageing reliability. To give you an idea of the length of time it takes to process and age 100 samples of various ageing structures refer to Appendix B.

Experienced fisheries personnel should demonstrate the following techniques to new staff or people outside the Ministry of Natural Resources (MNR) that are unfamiliar with calcified structures of fish.

Table 1. Structures to be collected from various fish species. Structures are listed in order of ageing reliability.

Species	Alive	Dead
Walleye and other Percids	• 1st three dorsal spines • scales	• otoliths • 1st three dorsal spines • opercular bones • scales
Lake Trout and other Salmonids	• 1st four leading pectoral rays • scales*	• otoliths • 1st four leading pectoral rays • scales*
Lake Whitefish and other Coregonids	• 1st four leading pectoral rays • scales	• otoliths • 1st four leading pectoral rays • scales
Northern Pike and Muskellunge	• scales	• cleithrum • scales
Lake Sturgeon	• pectoral fin ray	• pectoral fin ray
Smallmouth Bass and other Centrarchids	• 1st three dorsal spines** • scales	• otoliths • 1st three dorsal spines • opercular bones • scales
Burbot		• otoliths
Suckers	• 1st four leading pectoral rays • scales	• otoliths • 1st four leading pectoral rays • scales
Bullheads	• pectoral fin spine**	• otoliths • pectoral fin spine**
Smelt	• 1st four leading pectoral rays • scales	• otoliths • 1st four leading pectoral rays • scales

* As a single structure, scales are very poor indicators of age for trout

** The spine develops a medulary cavity in the centre after age 1

Otoliths

Studies have shown (Pannella 1971) that otoliths provide a method of assessing age with greater accuracy and precision than any other structure aged before.

In the skull of the fish, there are three paired calcareous structures. The largest pair of the three is the sagittae (Figure 1).

The smaller lapilli are slightly anterodorsal to the sagittae, and the asterisci are usually attached to the sagittae by a thin membrane. The sagittae are the structure typically used to assess the age of the fish. Collect both sagitta when sampling fish.

A fluid filled otolithic membrane called the succulus envelops the sagittae. The succulus **must** be removed before placing the otoliths in a 1.5 ml micro-centrifuge tube. Moisture on the otoliths promotes the growth of mold inside the tube. Another problem is the breakdown of the succulus creating an acidic environment. Otoliths are made of a calcium carbonate which will dissolve in an acidic environment. Rub them gently between the thumb and the forefinger or with a tissue to obtain clean, dry otoliths.

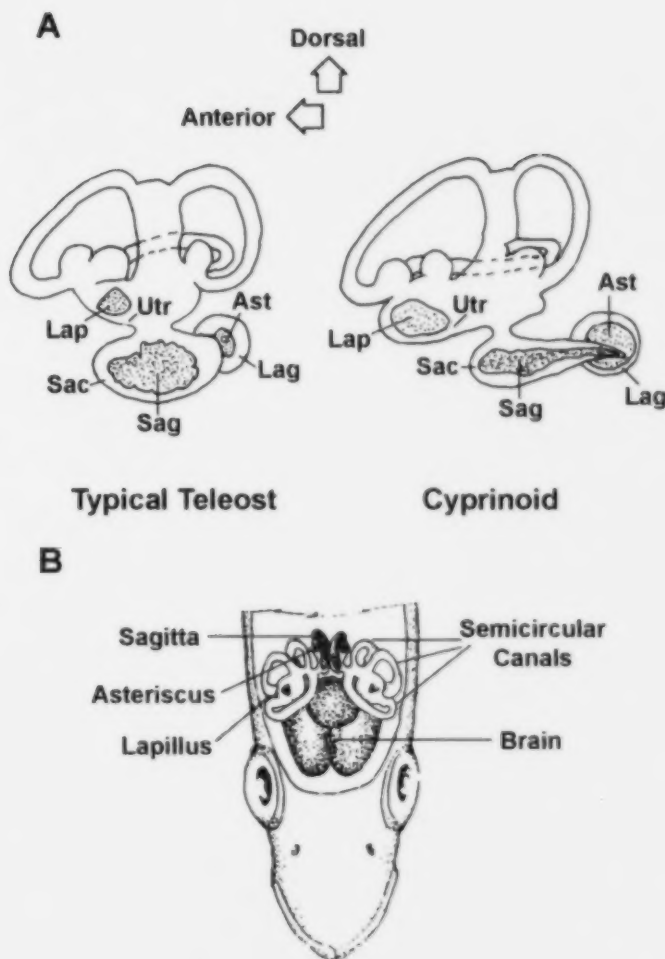


Figure 1. Anatomy of vestibular apparatus. (A) Otoliths within the labyrinth systems of representative teleost and ostariophyssean fishes.

(B) Dorsal view of the vestibular apparatus as it sits in a typical teleost. The top of head is cut away.

Ast = asteriscus
Lag = lagenar vestibule
Lap = lapillus
Sac = saccular vestibule
Sag = sagitta
Utr = utricular vestibule
(Secor *et al.* 1991).

The tubes can be stored two ways. The first method is placing the tube into the sample envelope directly and then stored in a sample envelope box. With this method approximately 30 samples fit into one box. Remember to number the lid and tube with a permanent marker to correspond with the sample envelope number in case of separation. In the second method, the tube is numbered with a permanent marker on the lid and tube to correspond to the sample envelope. It is then placed in a sample envelope box that has been fitted with a styrofoam form with holes that holds approximately 140 centrifuge tubes.

Both the centrifuge tubes and the styrofoam forms can be obtained free of charge for MNR staff from the Regional Fisheries Ageing Lab in Dryden.

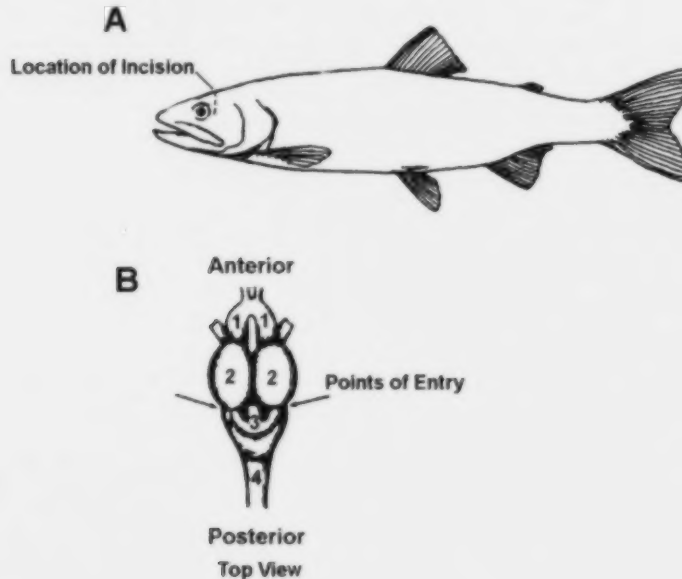
Otoliths stored unprotected in scale envelopes are more susceptible to breakage then when placed in tubes. They are naturally curved on the longitudinal axis. If they are compressed in the envelope with elastics, they will break. They cannot be cracked or sectioned properly in this condition.

There are two methods of extracting the sagittae or otoliths from fish skulls. Appendix C lists suppliers for various items that will be needed for the extraction of otoliths as well as other hard structures.

Dorsal Method

To remove the otoliths, a transectional cut is made through the dorsal cranial bones just behind the eyes and just ahead of the scaled areas of the nape (Figure 2). For small fish, a knife or strong pair of scissors may be used. For larger fish a fine saw such as a hacksaw can be used. Once the cranial bones are penetrated, grasp the head with one hand just in front of the cut and the trunk in the other hand, just behind the cut. The cut is bent and cracked open, widening the cut, exposing the cranial cavity and the brain. The otoliths are located just behind the cut in two depressions on the base of the cranial cavity. The soft tissue above the otoliths can be picked away with forceps (Casselman 1982). The forceps can then be used to remove the otoliths.

Figure 2. (A) Location of the dorsal incision for the removal of otoliths and (B) the top view of the brain and location where the forceps should be inserted. (Scarnecchia 1987)



Ventral Method

The dorsal method of cutting through the top of the skull is very time consuming especially when you have a large sample of fish. Often extensive searching is necessary to find the otoliths, especially if the otoliths are small as in salmonids. The ventral method is a faster alternative for removing otoliths. The otoliths are removed through the roof of the mouth and requires less than one minute and seldom requires a search.

To start the procedure, the gills and isthmus are removed (as when field dressing a fish) to expose the ventral (bottom) surface of the cranium. There, the parasphenoid bone is visible (Figure 3).

The otoliths are located in the bulbous shaped part of the posterior end of the parasphenoid. It is relatively soft and cartilaginous and can be cut easily. With a knife or pair of side cutters, make a lateral cut through the bulbous part, about three-fourths of the way through the bone and slightly twist the knife (Figure 4). If you wish to leave the gills attached, cut between the first and second pharyngeal (gill) arches to expose the otoliths on the ventral side of the cranium.

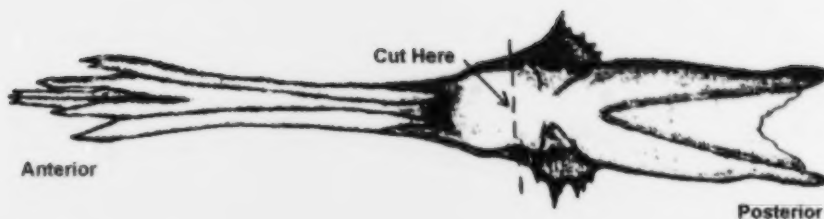


Figure 3. Schematic diagram of the ventral view of the parasphenoid bone. (Norden

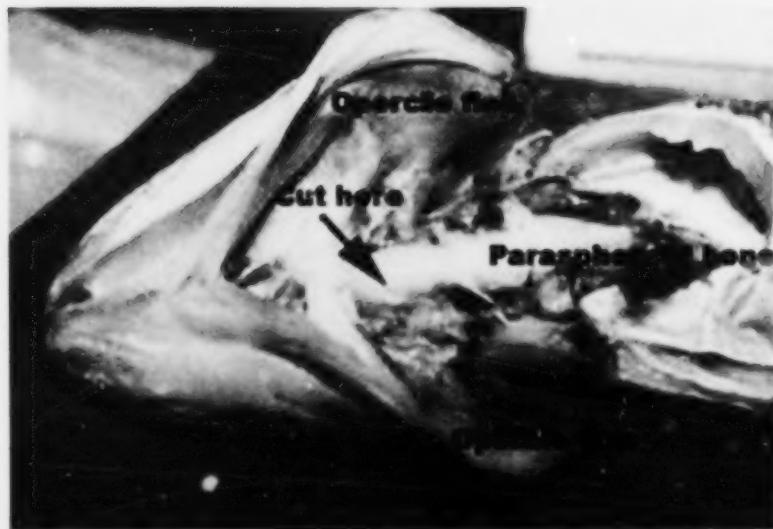


Figure 4. Location of incision on the ventral surface of the parasphenoid bone of a walleye. (Photo by Susan Mann)

Grasping the head with the left hand and the body with the right breaks the incision. Bend the two portions backwards until the cranium snaps. The exposed otoliths can then be removed easily with a pair of forceps. Once this technique is learned, otoliths can be routinely removed in about the same amount of time needed to take scales or fin ray samples. (Schneidervin and Huber 1986)

Cleithrum

Analysis of esocid cleithra can provide a rapid and relatively inexpensive method of monitoring exploitation and population structure by providing biological data not only on age, but also on size and growth rate, and to a limited extent sex and sexual maturity, and nutritional level (Casselman 1979).

Cleithrum can be removed by following the instructions as described by J.M. Casselman (1979) (Figure 5).

The fish is grasped behind the head with the right hand. The fingers of the left hand are hooked over the top of the head (Figure 5-A). Lifting the branchiostegal and opercular flaps (Figure 5-B) exposes the cleithrum. The thumb is pushed between the posterior edge of the cleithrum and the muscle and connective tissue (Figure 5-C); this separates the medial or inner surface of the cleithrum from the underlying soft tissue (Figure 5-D). The thumb is then moved along the inner surface towards the dorsal and posterior and towards the ventral and anterior (Figure 5-E) to loosen the bone. The thumb or index finger is then pushed through the connective tissue at the anterior of the cleithrum (Figure 5-F) and the cleithrum is pulled away from its dorsal articulation with the supracleithrum (Figure 5-G). When the dorsal tip of the cleithrum is

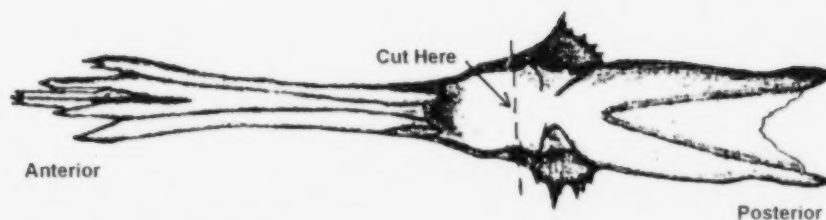


Figure 3. Schematic diagram of the ventral view of the parasphenoid bone. (Norden

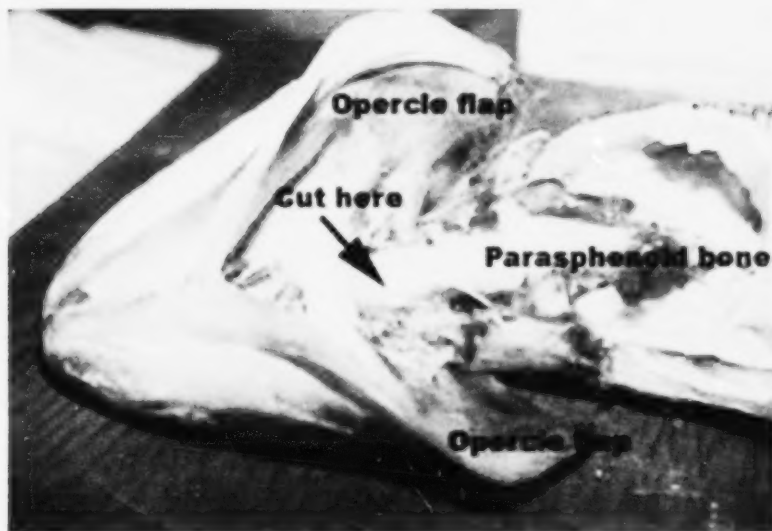


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Cleithrum

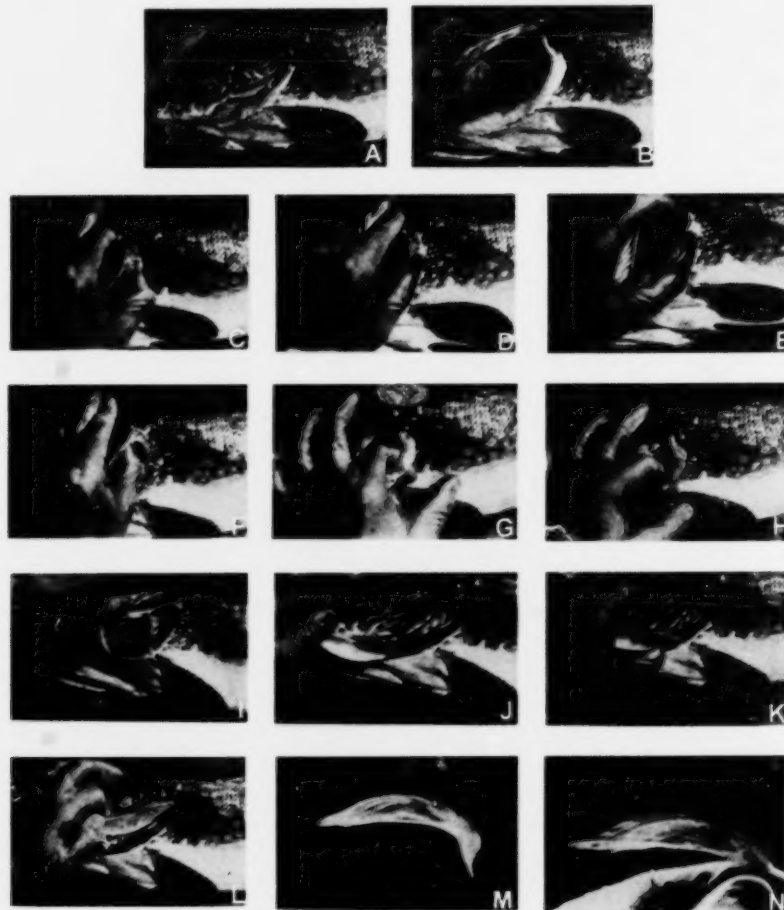
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released (Figure 5-H), it is grasped between the thumb and index finger. The cleithrum is then pulled out from the body and then towards the anterior (Figure 5-I). This exposes the anterior tip (Figure 5-J). **It is important during this procedure to pull the cleithrum strongly away from the body of the fish to avoid tearing or breaking the anterior tip and anterior growing edge.** The cleithrum is then pushed back over the thumb of the same hand (Figure 5-K) or over the thumb or index finger of the other hand. This peels away the muscle and connective tissue of the isthmus from the outer or lateral surface of the cleithrum (Figure 5-L). With this procedure the cleithrum is removed virtually free of muscle and connective tissue. Clean all excess flesh from the cleithrum.

Figure 5. Method of removing cleithra from northern pike and other esocids. (Casselman 1979)



When all the muscle and connective tissue has been removed, allow the cleithrum to dry before it is placed into the sample envelope. Moist and fleshy cleithrum invite insects and encourage the growth of mold. The cleithrum will also adhere to the inside of the envelope if not properly dried.

Figure 6 is an example of improperly sampled cleithrum. The flesh and pectoral fin should not be attached when placed into the sample envelope.

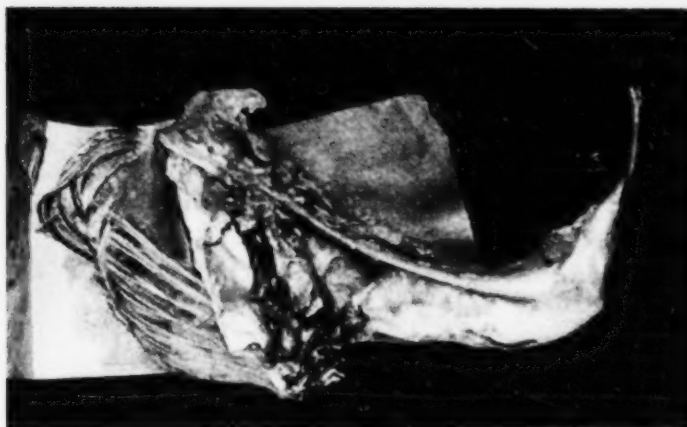


Figure 6. Photo of a cleithrum (right) that was improperly removed from a pike. Notice the pectoral fin ray is still attached as well as a large amount of muscle and connective tissue. (Photo by Susan Mann)

Opercular Bone

Not only does the opercular bone bear annuli for ageing purposes but can also be used to calculate the sizes of the fish at previous ages of their lives.

The opercular bone is easily and rapidly removed from the fish by slipping the point of a scalpel under the posterior edge of the bone between the opercular and the sub-opercular, as shown in Figure 7. The scalpel is then moved ventrally until it reaches the ventral end to the opercular. Twist to lift up the opercular. Grip the bone between the fingers and with a twist pull away from its point of articulation with the skull (Le Cren 1947). The sub-opercular bone may also be removed in the same fashion.

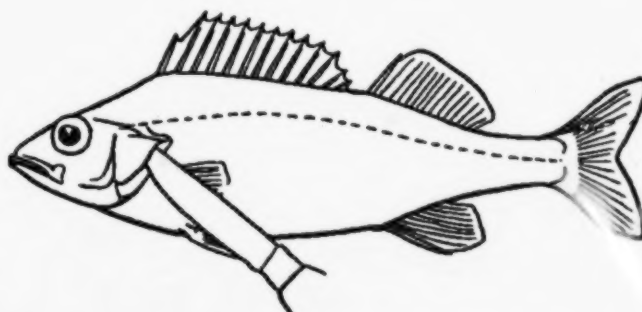
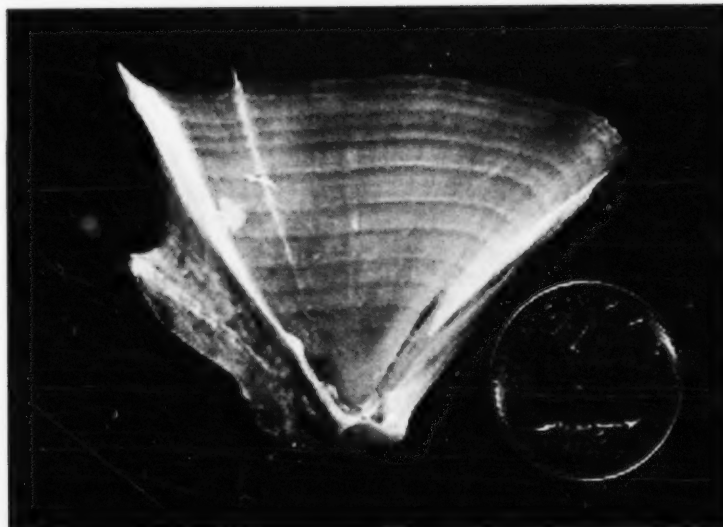


Figure 7. Diagram of a perch to show the location of the opercular bone. (Le Cren 1947)

When the bone is removed from the fish, ensure it is free of muscle and connective tissue. Place the cleaned and dry bone directly into the sample envelope. Again fleshy bones invite insects and encourage the growth of mold on the surface. The bones will also adhere to the inside of the envelope if not properly dried. All structures placed in the envelope should be indicated on the front. Figure 8 is a walleye opercular bone with an assessed age of 12 years.

This tissue requires very little preparation time and can also be measured for back calculations.

Figure 8. Photo of an opercular bone taken from a walleye. Age estimate is 15 years. (Photo by Susan Mann)



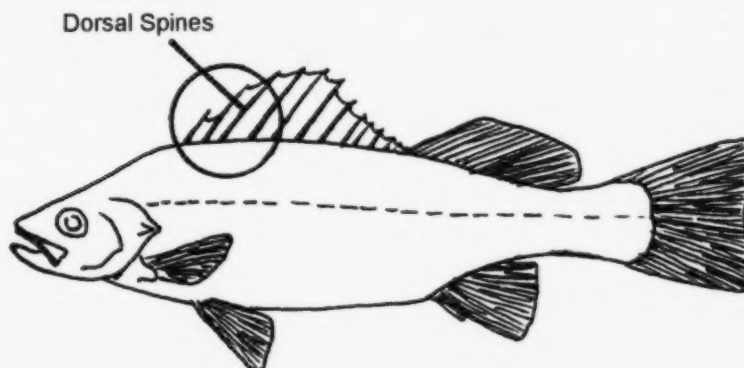
Spines

Spines can provide a fairly accurate age assessment for fish that are not sacrificed in a study. Spines are skeletal elements in the fins of bony fishes, characterized by: 1) a closed base; 2) distal radial always outside spine; 3) not separated into right and left halves; 4) unbranched distally; and 5) unsegmented (Summerfelt and Hall 1987).

When removing spines from percids and centrarchids, care should be taken to separate them as close to the body as possible with sharp side cutters. **The best section (to assess age) from the spine is obtained between the point of articulation and one centimetre above. If the spine is taken from above this zone, the assessed age might be inaccurate by one to several years.**

When collecting spines from dead fish, the first three dorsal spines should be taken from the anterior portion of the spine-rayed dorsal fin (Figure 9). When the fish is to be live-released, remove the third dorsal spine carefully so as not to tear the flesh and draw blood. If a long-term study is being conducted, consider the alternate spine removal method. For example, the third spine in the first year, the fifth spine in the second year, the seventh spine for the following year, etc. In this way, the field staff can easily see when the fish were recaptured in subsequent years (Mike Gauthier, personal communication).

Figure 9. Location of the dorsal spines used for age assessment on a spiny-rayed fish. (Drawing take from Scott and Crossman 1973)



To remove the left pectoral fin spine of bullheads and catfish, press the spine flat against the side of the body (Figure 10). This often requires the adductor muscles to be relaxed by gently tapping on the spine with pliers. Firmly grasp the base of the spine with pliers and twist counterclockwise (clockwise for the right side) while pushing towards the body. This method facilitates dislocation from the articulating process (Blouin and Hall 1990). Place the spine, sharp point in first, into the sample envelope and allow it to dry.

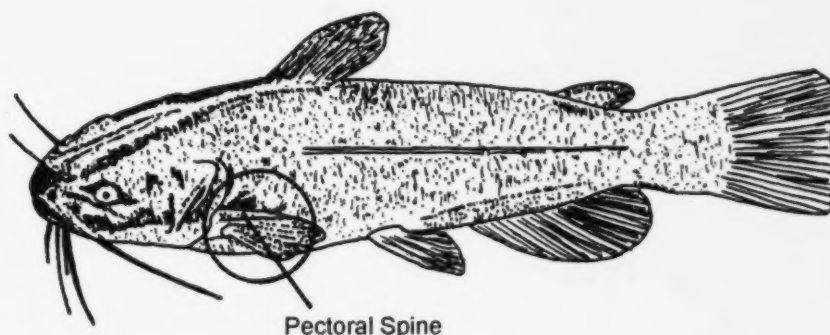


Figure 10. Location of the pectoral fin spine used for age assessment on bullheads and catfish. (Drawing taken from Scott and Crossman 1973)

Fin Rays

Fin rays are skeletal elements in the fins of bony fishes, characterized by: 1) an open base; 2) distal radial element with the open base; 3) right and left halves either separate or with a mid-sagittal suture; 4) branched distally; and 5) always segments. Usually, criteria 4) and 5) hold true; however, there are exceptions (Summerfelt and Hall 1987).

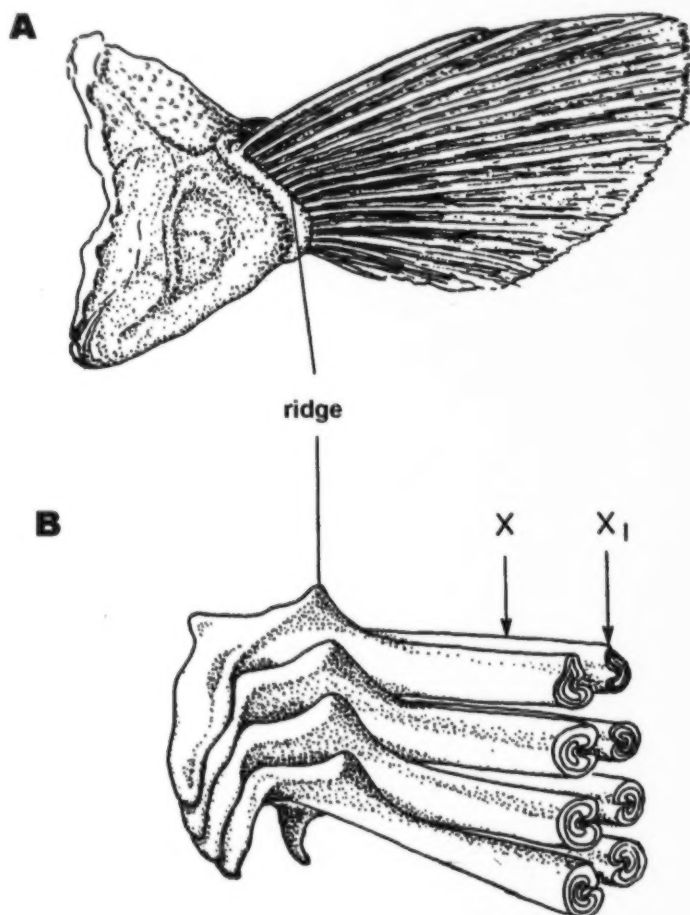
As in spines, the pectoral fin ray must be separated as close to the body as possible where annulus assessment is easier resulting in more reliable ages (Figure 11). For live-release, do not remove the fin rays too close to the body or bleeding may occur and jeopardize the survival of the fish (Mike Gauthier, personal communication).

The best sections for age assessment are taken from just above the ridge. Take only the first four leading marginal rays and not the whole structure. Be sure to include the entire length of the rays so the structure can be secured in the chuck of the isomet saw. Wipe the mucus from the sample and place it (distal end first) into a waxpaper insert approximately 75 mm x 75 mm (3 inches x 3 inches). Fold the waxpaper in half to hold the fin ray. It does not have to be in a series of complicated folds. This insert will stop the fin ray from sticking to the sample envelope. If the fin ray is placed in the envelope without an insert, the fin ray will adhere to the fibers of the envelope. This could subsequently damage the fin ray and/or envelope along with any written data when the ray is torn out.

Scales

Scales from young fish are very useful in age determination, as well as back calculations for the study of growth for individual fish. As fish grow older, however, there are periods of little or no scale growth. If this period extends longer than twelve months, the scale age will underestimate the true age of the fish (Beamish and McFarlane 1987).

Figure 11. (A) shows the position where the fin should be removed by cutting through or close to the ridge. (B) shows the two fin ray elements for each fin ray (Beamish 1981)



Scales are collected from the left side of the body. Scales from spiny-rayed fish should be removed from below the lateral line and posterior to the insertion of the pectoral fin (Figure 12A). Scales from soft-rayed fish should be removed from above the lateral line and below the dorsal fin (Figure 12B).

Wipe mucus and dirt from the area to be sampled with a knife. The knife should be carefully cleaned either by wiping on a cloth or rinsing in a bucket of water. This prevents contamination of the sample with scales of a previous fish and ensures a clean scale sample. With the tip of the knife, pull approximately six to 10 scales from the body and place in a scale sample envelope. Note that dirty scales do not imprint clearly onto acetate slides and delays the procedure if the scales need to be cleaned.

Do not take scales from those areas of the body where previous damage has occurred and scale regeneration is evident. Depending on the degree of regeneration, the first few annuli may not be present.

REMOVAL OF FISH SCALES USED FOR AGEING

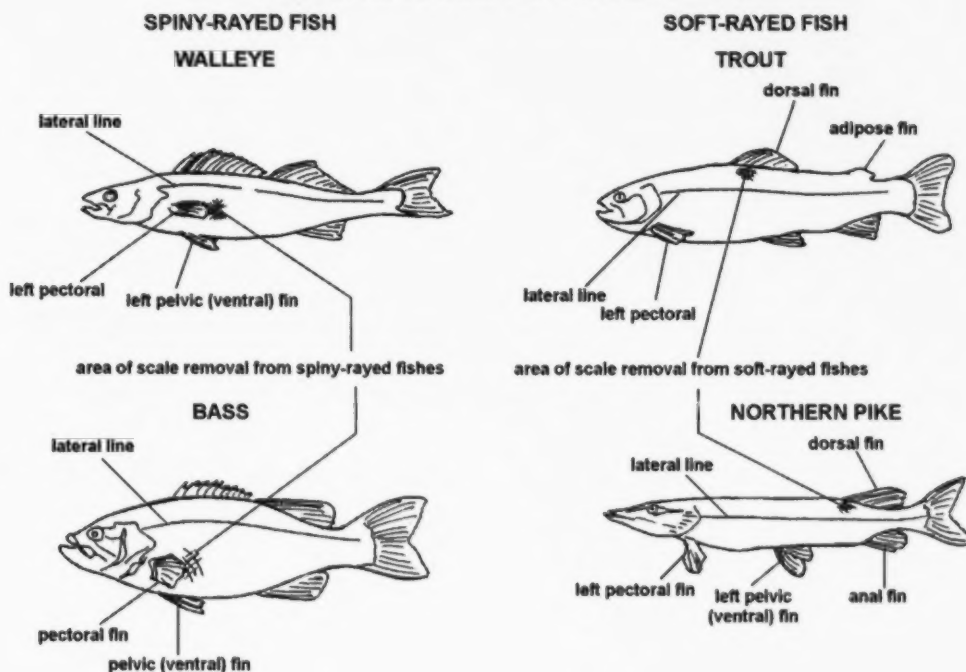


Figure 12. Location of scale removal from walleye and other percids; bass and other centrarchids; trout and other salmonids; and pike and other esocids. (Drawings by Susan Mann)

Data Records

Proper recording of biological data on the sample envelope is imperative (i.e. total and fork length in millimetres, weight in grams, sex, species code, sexual maturity, gear type, capture date, etc.) for the ageing procedure and data entry. Record the date as year.month.day (i.e. 03.07.09). Use the metric unit of measurement throughout the program to avoid confusion. All of the calcified structures sampled for the fish should be identified on the sample envelope. The structure names can be written out in full or coded from the Ontario Fisheries Information System (OFIS) Data Dictionary (Table 2).

AGEST Age Structures Sampled (Fish Age Structures Sampled Code)

Code listing calcified structures collected for the purpose of age determination. As many as 4 structures may be listed.

If a wider field is required, make a user defined field called XAGEST.

- | | |
|-------------------------|-------------------|
| 0 No structures sampled | A Otolith |
| 1 Scales (side unknown) | B Operculum |
| 2 Scales (left side) | C Sub-operculum |
| 3 Scales (right side) | D Cleithrum |
| 4 Pectoral ray | E Centrum |
| 5 Pectoral spine | F Branchiostegal |
| 6 Pelvic ray (ventral) | G Other |
| 7 Dorsal spine | X More structures |

E.G. 1 ⇒ Scales only
 16 ⇒ Scales and pelvic ray
 27AX ⇒ Scales, dorsal spine, otolith, and more structures

Table 2. AGEST section of the OFIS Data Dictionary

Sample envelopes should be organized before they are sent to the lab, so that the structures can be processed and aged quickly. Be sure that the flap of the envelope is folded over but not tucked in or glued shut. There have been several instances where the contents have emptied out while the flap was tucked in. Envelopes should not be wrapped in elastic bands as the structures may become compressed and broken. Try to keep species separate by box with a label stating the waterbody name, species, FISHNET project code, MNR District office name, and a contact name and number.

Samples should be accompanied by an electronic version of data files (i.e. FISHNET, Excel, or Access). A copy of the data files will be kept at the lab and the original sent back to the MNR District office once the samples have been aged. This form has columns for the calcified structure age. If your work unit requires a different format than this, space should be left for an assessed age to be entered. The form can be obtained via email from Susan.Mann@mnr.gov.on.ca.

Shipping

Boxes of samples should be secured by a protective wrapping that will withstand the rigours of shipping by mail or courier service. Enclose a covering letter (you'd be surprised at the number of people that don't) that explains what you would like done with your samples i.e. Age 1, 2, or 3 structures, age as soon as possible, sub-sample structures, etc.

The shipping address for the Regional Fisheries Ageing Lab in the Northwest Region is:

Ministry of Natural Resources
Northwest Science and Information
479 Government Road
Dryden, ON P8N 3K9
Attention: Susan Mann, Regional Ageing Laboratory

The Regional Fisheries Ageing Lab is located on the main floor of the Ontario Government Building, Hwy 17 on the west side directly beside the loading dock.

Summary

1. Calcified structures handled with respect will generally give a truer estimate of the age of a fish and therefore a more accurate picture of the health of a population than damaged structures.
2. Otoliths are calcified structures, easily damaged in an acidic environment. The otolith sac or succulus must be removed to prevent the disintegration of the outer annuli.
3. Remove all flesh from the cleithrum and operculum before placing them in a sample envelope. Care should be taken to prevent the breaking of the anterior tip and the anterior growth edge of the cleithrum and operculum.
4. Spines and fin rays should be removed from the fish as close as possible to the body to prevent the loss of the first annulus.
5. Remove cleaned and unregenerated scales from specified areas of the fish.
6. Keep consistent data records. Organize the samples before sending them to the Regional Fisheries Ageing Lab to reduce processing time. Parcel the samples to avoid any damage or breakage.

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Appendix A

Examples of ageing error using the scale method (Beamish and McFarlane 1987).

Species	Reference	Comment
<i>Salvelinus namaycush</i>	Simard and Magnin 1972	Scale growth slows down at maturity while otoliths continue to grow in relation to length.
<i>Salvelinus namaycush</i>	Dutil and Power 1977	Scale age was generally less than otolith age and could underestimate ages by 40%.
<i>Coregonus clupeaformis</i>	Mills and Beamish 1980	Fin ray ages were older than scale ages for slower growing fish.
<i>Esox masquinongy</i>	Harrison and Hadley 1979	Fish older than 9 could not be aged from scales while ages of 19 were obtained using cleithra.
<i>Catostomus commersoni</i>	Beamish and McFarlane 1983	Tagging studies confirmed that scale age substantially underestimates true age and that fin ray ages are more reliable.
<i>Perca flavescens</i>	Schmitt and Hubert 1982	For older perch cleithra provides more reliable ages than scales.
<i>Stizostedion vitreum vitreum</i>	Campbell and Babaluk 1979	Scale method underestimates age of older fish in unexploited populations. Dorsal fin spines were recommended for age determination.
<i>Stizostedion vitreum vitreum</i>	Belanger and Hogler 1982	Otolith or dorsal spine ageing methods should be used as an occasional check for scale ages.

Appendix B

Comparison of preparation and ageing time for 100 samples of each structure at the Northwest Region Ageing Laboratory.

Structure	Number Of Structures/hour
Scales	25
Fin rays	14
Spines	17
Cleithra	15
Otoliths (grind and polish method)	9
Otoliths (crack and burn method)	23

Appendix C

Supplies and Suppliers

Supplies	Size	Source	Item #	Price Range
Side cutters		Canadian Tire		\$6 - \$10
Hack saw		Canadian Tire		\$10 - \$15
Forceps		Fisher Scientific	08-953E	\$13.00
Microcentrifuge tubes (pkg of 500)	1.5 ml	Fisher Scientific	05-407-10	\$390.00
		VWR Canlab	53550-698	\$296.00

Source	Address	Telephone Number
Fisher Scientific	111 Scotia Court Whiteby, ON L1N 6J6	1-800-234-7437
VWR Canlab	77 Enterprize Drive North London, ON N6N 1A5	1-800-932-5000

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